pET-43.1 Ek/LIC Vector

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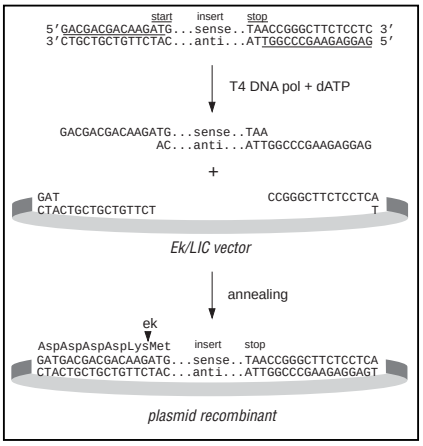
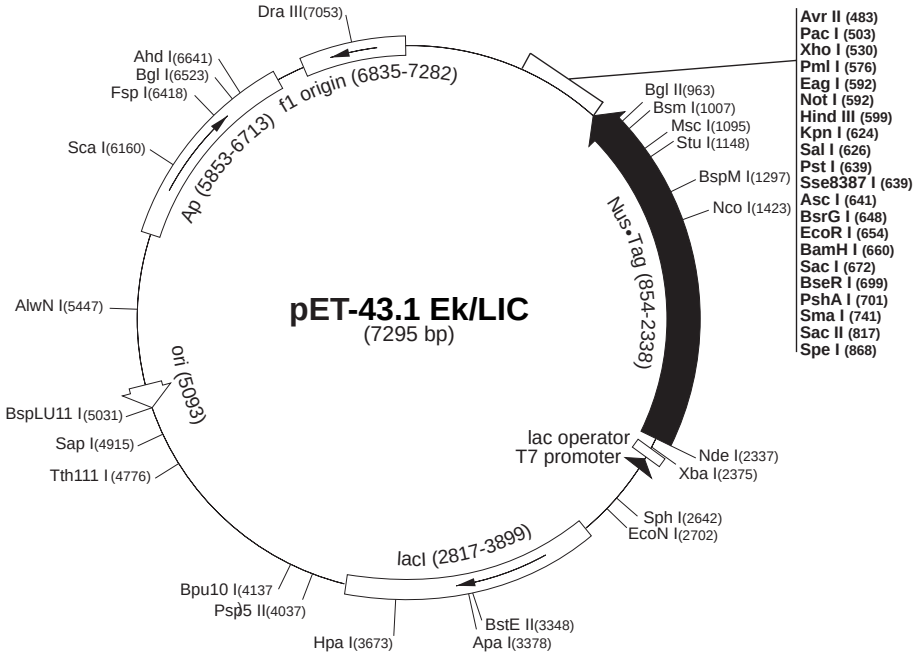
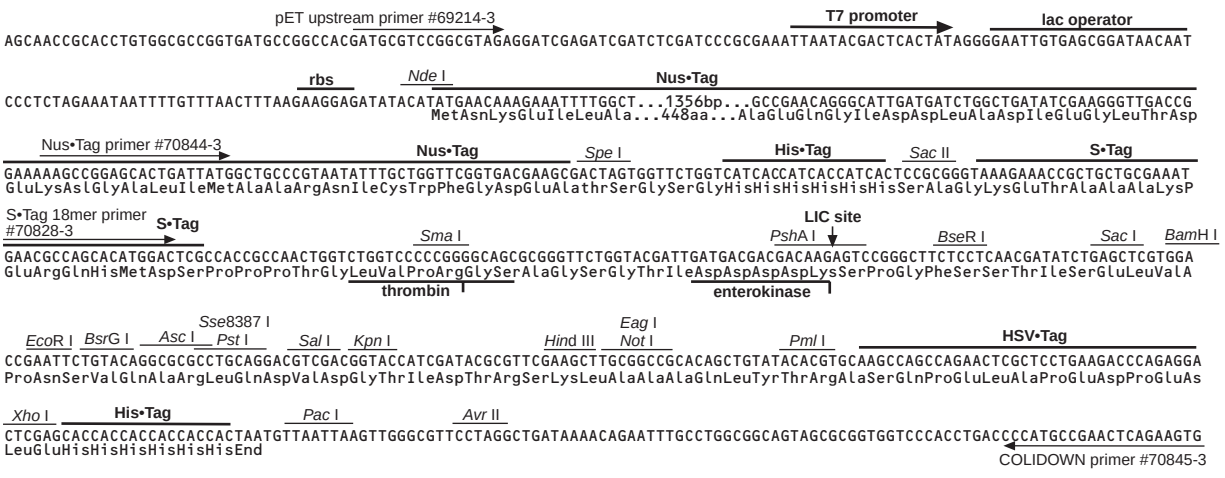
The pET-43.1 Ek/LIC vector is prepared for rapid, directional cloning of PCR-amplified DNA for high-level expression of polypeptides fused with N-terminal Nus•Tag™, His•Tag® and S•Tag™ sequences. Using specifically designed primers for amplification and the pET-43.1 Ek/LIC Vector Kit (Cat. No. 71072-3), inserts can be efficiently cloned without the need for restriction digestion or ligation. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circle map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single stranded DNA that corresponds to the coding strand. Therefore, single stranded sequencing should be performed using the COLIDOWN primer (Cat. No. 70845-3). Vector encoded sequence can be completely removed when cloning into the Ek/LIC site (as shown below left) by cleaving the fusion protein with enterokinase.

pET-43.1 EK/LIC sequence landmarks

T7 promoter	2410-2426
T7 transcription start	2409
Nus•Tag coding sequence	854–2348
His•Tag coding sequence	821–838
S•Tag coding sequence	767–811
Multiple cloning sites (*Bse*R I-*Xho* I)	699–530
HSV•Tag coding sequence	536–571
His•Tag coding sequence	512–529
T7 terminator	26–72
lacI coding sequence	2817–3899
pBR322 origin	5093
bla (Ap) coding sequence	5854–6714
f1 origin	6835–7282







pET-43.1 Ek/LIC cloning/expression region

pET-43.1 Ek/LIC Restriction Sites

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Enzyme	# Sites	Locations				
AatII	2	633	2208			
AccI	4	580	627	1549	4801	
AciI	99					
AflIII	4	575	608	3167	5031	
AhdI	1	6641				
AluI	27					
Alw26I	8	371	2864	3269	3395	3782
		4672	5798	6575		
AlwI	20					
AlwNI	1	5447				
ApaI	1	3378				
ApaLI	4	3147	4845	5345	5969	
ApoI	7	466	654	788	2323	3442
		6855	6866			
AscI	1	641				
AvaI	2	530	739			
AvaII	7	440	745	3719	4037	4316
		6277	6499			
AvrII	1	483				
BamHI	1	660				
BanI	10	620	1470	2489	2510	2624
		3087	3806	3936	6688	7090
BanII	5	672	2551	2565	3378	7128
BbsI	5	538	1228	3313	3652	4149
BbvI	28					
BcgI	3	3493	4608	6103		
BclI	2	1953	3181			
BfaI	10	70	484	849	2376	4010
		4045	5526	6448	6783	7204
BglI	1	6523				
BglII	1	963				
BpmI	4	3005	3494	4558	6572	
Bpu10I	1	4137				
Bpu1102I	2	80	1298			
BsaAI	3	576	4783	7053		
BsaBI	3	2440	2450	4228		
BsaHI	9	200	630	2205	2490	2511
		2625	3124	3807	6101	
BsaI	2	371	6575			
BsaJI	17					
BsaWI	8	2	1596	3486	3989	4220
		5237	5384	6345		
BseRI	1	699				
BsgI	4	1976	3018	3218	4191	
BsiEI	10	595	1135	1273	1809	2190
		3952	4947	5371	6123	6272
BsiHKAI	11					
BsII	26					
BsmBI	2	3782	4672			
BsmFI	5	453	758	2628	4302	7268
BsmI	1	1007				
Bsp1286I	15					
BspEI	2	2	4220			
BspLU11I	1	5031				
BspMI	1	1297				
BsrBI	6	249	1343	2396	4964	5798
		7197				
BsrDI	6	1093	1474	3214	3580	6407
		6581				
BsrFI	9	1047	1403	1467	1919	2477
		2486	2853	6556	7154	
BsrGI	1	648				
BsrI	29					
BssHII	5	641	1567	1759	2248	3578
BssSI	4	665	1639	5204	5972	
Bst1107I	2	581	4802			
BstEII	1	3348				
BstXI	3	2969	3098	3221		
BstYI	13					
Cac8I	52					
ClaI	5	615	1524	1602	2190	2444
CviJI	113					

Enzyme	# Sites	Locations				
DdeI	15					
DpnI	42					
DraI	3	6063	6755	6774		
DraIII	1	7053				
DrdI	3	4724	5139	7008		
DsaI	4	814	984	1423	2604	
EaeI	8	592	991	1093	1380	2475
		2607	3841	6248		
EagI	1	592				
EarI	3	2785	4915	5842		
Ecl136II	1	670				
Eco47III	2	2572	4285			
Eco57I	4	532	1846	5579	5975	
EcoNI	1	2702				
EcoO109I	3	53	2600	4037		
EcoRI	1	654				
EcoRII	13					
EcoRV	3	677	931	1522		
EheI	4	2491	2512	2626	3808	
FauI	20					
Fnu4HI	52					
FokI	14					
FspI	1	6418				
HaeII	15					
HaeIII	31					
HgaI	15					
HhaI	59					
HincII	4	628	920	1315	3673	
HindIII	1	599				
HinfI	16					
HpaI	1	3673				
HphI	29					
KpnI	1	624				
MaeIII	22					
MboII	25					
MluI	2	608	3167			
MnlI	29					
MscI	1	1095				
MseI	33					
MslI	11					
MspA1I	13					
MspI	42					
MwoI	47					
NarI	4	2490	2511	2625	3807	
NciI	20					
NcoI	1	1423				
NdeI	1	2337				
NgoAIV	2	2477	7154			
NlaIII	28					
NlaIV	25					
NotI	1	592				
NruI	3	1230	1655	2279		
NspI	4	2642	4376	4668	5035	
NspV	2	604	2265			
PacI	1	503				
PflMI	2	778	2749			
PleI	12					
PmlI	1	576				
PshAI	1	701				
Psp1406I	6	237	2829	4356	6039	6412
		6838				
Psp5II	1	4037				
PstI	1	639				
PvuI	3	1273	2190	6272		
PvuII	4	586	3767	3860	4622	
RcaI	4	2565	5751	5800	5832	
RsaI	8	622	650	721	976	2198
		3314	4837	6160		
SacI	1	672				
SacII	1	817				
SalI	1	626				
SapI	1	4915				

Enzyme	# Sites	Locations				
Sau3AI	42					
Sau96I	20					
ScaI	1	6160				
ScrFI	33					
SfaNI	23					
SfcI	7	635	2314	2409	5296	5487
		6395	7272			
SgrAI	2	1919	2486			
SmaI	1	741				
SpeI	1	848				
SphI	1	2642				
Sse8387I	1	639				
SspI	2	877	6845			
StuI	1	1148				
StyI	4	57	483	1423	2119	
TaiI	25					
TaqI	28					
TfiI	4	3846	4081	4585	5006	
ThaI	52					
TseI	28					
Tsp45I	10	859	1745	2132	3348	4470
		4683	4778	6168	6379	7226
Tsp509I	23					
TspRI	16					
Tth111I	1	4776				
VspI	4	2424	3852	3911	6466	
XbaI	1	2375				
XcmI	3	3023	3539	3557		
XhoI	1	530				
XmnI	3	2271	4589	6041		

Enzymes that do not cut pET-43.1 Ek/LIC:

AflII	Bsu36I	FseI	MunI	NheI	NsiI	PinAI
PmeI	RsrII	SanDI	SexAI	SfiI	SgfI	SnaBI
SrfI	SunI	SwaI				